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Primer on the Rheumatic Diseases

EDITION 12

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ARTHRITIS FOUNDATION®

Take Control. We Can Help.™

Published by the Arthritis Foundation
1330 West Peachtree Street
Atlanta, Georgia 30309

Library of Congress Catalog Card Number: 2001090001
ISBN: 0-912423-29-3

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Printed in Canada

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MEDIATORS OF INFLAMMATION, TISSUE DESTRUCTION, AND REPAIR
B. Growth Factors and Cytokines

Cytokines are small molecular-weight proteins that mediate communication between cells. The generic term "cytokine" includes colony-stimulating factors, growth factors, interleukins, and interferons. The terminology of these molecules is confusing because it is largely historically based rather than functionally based. For example, some interleukins primarily serve to regulate cell growth and differentiation, whereas some growth factors have other major properties.

Cytokines carry out their functions largely in the immediate pericellular environment, either in an autocrine fashion, influencing the same cell that produced the cytokine, or in a paracrine fashion, influencing adjacent cells. Cytokines bind to specific plasma-membrane receptors on target cells. Subsequent activation of secondary messenger pathways or other intracellular mechanisms leads to alterations in transcription and production of proteins.

Cytokines are involved as mediator molecules in normal biologic processes. These physiologic functions include growth and differentiation of hematopoietic, lymphoid, and mesenchymal cells, as well as orchestration of host defense mechanisms. Multiple cytokines operate as a network in a redundant, overlapping, and synergistic manner. However, the cytokine network is largely self-regulating, and pathophysiologic consequences may result from the unregulated action or inappropriate production of particular cytokines.

This chapter's review of cytokines uses arbitrary groupings based on primary functions. These categorizations include colony-stimulating factors, growth and differentiation factors, immunoregulatory cytokines, and proinflammatory cytokines (Table 4B-1). This chapter also emphasizes the relevance of each cytokine to the function of lymphoid and inflammatory cells, particularly its possible role in rheumatic diseases. The self-regulatory nature of the cytokine network also is discussed, in a review of mechanisms that inhibit the effects of cytokines.

Colony-Stimulating Factors

Colony-stimulating factors (CSFs) and related molecules function primarily as hematopoietic growth factors (1). However, this group of cytokines also demonstrates profound effects on mature lymphocytes, neutrophils, monocytes, and macrophages. It is in these latter effects that CSFs may play significant roles in rheumatic diseases.

Granulocyte-macrophage CSF (GM-CSF) and interleukin (IL)-3 potentiate the growth of numerous early bone marrow precursor cells, whereas erythropoietin influences only erythroid precursor cells. Each of these factors is preceded in its

TABLE 4B-1
Functional Classification of Cytokines

Colony-stimulating factors (CSFs)
GM-CSF (granulocyte-macrophage CSF)
G-CSF (granulocyte CSF)
M-CSF (macrophage CSF or CSF-1)
IL-3 (interleukin 3)
Erythropoietin
Growth and differentiation factors
PDGF (platelet-derived growth factor)
EGF (epidermal growth factor)
FGF (fibroblast growth factor)
TGF-β (transforming growth factor β)
ODF (osteoclast differentiation factor)
Immunoregulatory cytokines
IFN-γ (interferon γ)
IL-2, 4, 5, 7, and 9-18
Proinflammatory cytokines
TNF-α (tumor necrosis factor α)
IL-1, 6, and 8
Anti-inflammatory cytokines and growth and differentiation factor inhibitors
IL-1Ra (interleukin-1 receptor antagonist)
IL-4, 10, and 13
OPG (osteoprotegerin)

actions by other stem-cell growth and differentiation factors. The effects of GM-CSF and IL-3 are enhanced by the presence of IL-1 and IL-6. In contrast, granulocyte CSF (G-CSF) influences the growth and function of mature neutrophils, and macrophage CSF (M-CSF) serves the same role for monocytes and macrophages. GM-CSF, G-CSF, and M-CSF are produced by monocytes, fibroblasts, and endothelial cells; in addition, GM-CSF is produced by T lymphocytes.

In addition to its effects as a growth factor, GM-CSF influences the function of mature cells of the granulocytic and monocytic lineages. GM-CSF primes neutrophils, eosinophils, and basophils to respond to triggering agents with enhanced chemotaxis, oxygen radical production, and phagocytosis. GM-CSF also enhances eosinophil cytotoxicity and stimulates basophil release of histamine. These multiple effects of GM-CSF serve to heighten the inflammatory response in acute rheumatic diseases.

GM-CSF also influences diverse functions of monocytes and macrophages, leading to an enhanced ability of these cells to present antigen and induce an immune response. These functions include increased expression of membrane-bound IL-1a and of class II molecules of the major histocompatibility complex (MHC). Monocytes differentiated in the presence of GM-CSF produce more IL-1 receptor antagonist (IL-1Ra), possibly leading to an inhibition of IL-1 effects. These properties of GM-CSF illustrate an essential principle of cytokine biology: A single cytokine may exhibit activating and suppressing effects simultaneously.

The possible role of GM-CSF in rheumatic diseases is illustrated best by rheumatoid arthritis (RA). This is the only human disease in which both GM-CSF protein and mRNA are known to be localized in the damaged tissue. GM-CSF also is present in RA synovial fluids. The enhanced expression of class II MHC molecules observed on macrophages from RA synovial tissue may be secondary to the effects of GM-CSF.

Interleukin 1 and tumor necrosis factor α (TNF-α) stimulate monocytes, fibroblasts, and endothelial cells to produce more GM-CSF. Some investigators believe that chronic inflammation and tissue destruction in some people with RA may result from a cytokine-mediated self-perpetuating cycle without a significant component of continuous T-cell activation.

Growth and Differentiation Factors

A number of cytokines exhibit as their major property a growth enhancement of specific cell types. These cytokines include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factor β (TGF-β). Other cytokines, such as the CSFs and many of the interleukins, also promote growth.

PDGF primarily is a product of platelets but also is produced by macrophages, endothelial cells, and other cells. There are three different forms of PDGF and two different PDGF receptors. The biologic properties exhibited by PDGF vary with the form synthesized by a particular cell and the predominant receptor expressed on a target cell. EGF is found throughout the body and is a potent angiogenic factor, as is FGF. Two main forms of FGF exist, but many structural variants have been described. Both EGF and FGF induce the growth and proliferation of a variety of mesenchymal and epithelial cells, and FGF also may stimulate osteoclasts in the rheumatoid synovium.

The marked proliferation of synovial fibroblasts that occurs in RA synovium probably is secondary to the effects of PDGF, EGF, and FGF. Tissue fibrosis present in other diseases, such as scleroderma, may also be due in part to PDGF, EGF, and FGF. The greatly enhanced growth of new capillaries that characterizes synovitis in RA likely is a result of multiple factors, including FGF, IL-8, TNF-α, and vascular endothelial growth factor (VEGF). These growth factors are all present in synovial fluids of people with RA and are produced by synovial macrophages.

The importance of angiogenesis in the pathophysiology of rheumatoid synovitis has been reviewed (2). Both angiogenic inducers and inhibitors are present in the joint fluid and tissue, with the balance between the two sets of factors deter-

mining whether new capillary growth will predominate. Compared with cells from controls, peripheral blood monocytes from RA patients produce greatly enhanced amounts of VEGF in response to TNF-α stimulation, and synovial lining macrophages in this disease contain large amounts of VEGF mRNA. In fact, when IL-1 and TNF-α in rheumatoid synovial membrane cultures were neutralized with IL-1Ra and a monoclonal antibody to TNF-α, VEGF release was reduced by approximately 50%. The administration of angiogenesis inhibitors effectively reduced inflammation and tissue destruction in two animal models of arthritis.

The last and most important growth factor to be discussed, TGF-β, has both potent proinflammatory and anti-inflammatory effects (Table 4B-2) (3). TGF-β exhibits many biologic properties, but its most important effects in rheumatic diseases include recruitment of monocytes into tissues, dampening of lymphocyte and macrophage functions, and stimulation of tissue fibrosis. More than any other cytokine, TGF-β exemplifies the apparent paradox of simultaneously enhancing inflammatory responses and promoting repair. In general, TGF-β is stimulatory toward resting or immature cells and when confined to local environments, but is inhibitory toward differentiated cells and when present systemically.

TGF-β is the major member of a family of molecules that may serve important roles in embryogenesis of mesenchymal tissues. Many cells in the adult contain mRNA for TGF-β, but macrophages and platelets are the main sources of protein. TGF-β is released in a latent form and must be activated in tissues, presumably by proteases.

The presence of other cytokines in a particular tissue may influence whether TGF-β enhances or inhibits growth and differentiation in fibroblasts. For example, TGF-β and EGF together may suppress the growth of particular types of fibroblasts, whereas the combination of TGF-β and PDGF stimulates their growth. The enhancing effects of TGF-β on cell growth may be mediated by inducing PDGF production. TGF-β induces production of collagen and fibronectin in fibroblasts; however, interferon gamma (IFN-γ) and TNF-α both oppose this effect on collagen synthesis. In the presence of PDGF, EGF, or FGF, TGF-β inhibits fibroblast production of collagenase and other neutral proteases, while enhancing production of inhibitors of these enzymes.

TGF-β is thought to be responsible, at least in part, for tissue fibrosis in a variety of human diseases, including scleroderma, pulmonary fibrosis, and chronic glomerulonephritis. Infiltrating monocytes in skin and organ lesions of scleroderma contain TGF-β mRNA. TGF-β protein has been found in skin lesions adjacent to fibroblasts and areas of fibrosis. The observation that IFN-γ inhibits TGF-β–induced collagen production by fibroblasts in vitro led to clinical trials of IFN-γ in people with scleroderma. Unfortunately, no clear clinical benefit of IFN-γ administration was observed in established dermal fibrosis in this disease.

TGF-β exhibits potent effects on monocytes and lymphocytes. TGF-β is the strongest known chemotactic agent for monocytes. In addition, TGF-β enhances expression of Fc receptor III on these cells but may block production of cytokines. It may promote inflammation; injection of TGF-β into rat joints leads to an influx of monocytes, with swelling, redness, and eventual hyperplasia of synovial fibroblasts. However, the net effects of TGF-β on macrophage function are suppressive and include a decrease in human leukocyte antigen (HLA)-DR expression and a deactivation of H_2O_2 production. Overall, TGF-β is thought to call monocytes into an acutely inflamed tissue, contribute to fibroblast proliferation, and then promote fibrosis.

TGF-β exhibits immunosuppressive effects on B cells, T cells, and natural killer (NK) cells. TGF-β inhibits IL-1–induced T-cell proliferation, B-cell growth, and immunoglobulin (Ig) production after stimulation by IL-

TABLE 4B-2
Roles of TGF-β in Human Inflammatory Diseases

Proinflammatory effects of TGF-β
Locally stimulates resting or immature monocytes, lymphocytes, and chondrocytes
Recruits monocytes into inflammatory tissues
Enhances cell growth primarily through induction of PDGF production
Stimulates collagen production by fibroblasts, with subsequent tissue fibrosis
May be involved in inducing fibrosis in scleroderma and interstitial lung disease
Stimulates angiogenesis
Anti-inflammatory effects of TGF-β
Inhibitory towards differentiated cells and when present systemically
Leads to a decrease in monocyte HLA-DR expression and in H_2O_2 production
Inhibits B-cell and T-cell growth and proliferation and is generally immunosuppressive
Blocks NK-cell activation induced by IFN-γ
Inhibits IFN-γ–induced collagen production in fibroblasts

TGF, transforming growth factor; PDGF, platelet-derived growth factor; HLA-DR, human leukocyte antigen-DR; NK, natural killer; IFN, interferon.

2 and IL-4. IFN-γ–induced NK-cell function is opposed by TGF-β. The immunosuppression that occurs in streptococcal cell-wall–induced arthritis in rats is thought to be secondary to TGF-β, and a similar situation may exist in the joints of people with RA.

Bone resorption in RA may be secondary to the effects of osteoclasts under the influence of osteoclast differentiation factor (ODF) (4). ODF may be synthesized by synovial fibroblasts and T cells, and it acts with M-CSF to induce differentiation of synovial monocytes into osteoclasts. A specific inhibitor of ODF has been described, osteoprotegerin (OPG), which binds to both soluble and membrane ODF to prevent interaction with cell-surface receptors on osteoclast precursors. Treatment of animal models of arthritis with OPG prevents cartilage and bone destruction but does not reduce inflammation. The balance between ODF and OPG in the rheumatoid synovium may influence the relative degree of bone erosion. These exciting new findings establish a foundation for novel therapeutic approaches to inhibit the effects of osteoclasts, not only in RA but also in osteoporosis.

Thus, growth and differentiation factors may be primarily responsible for fibroblast proliferation, angiogenesis, and bone resorption in many human chronic inflammatory diseases. In addition, TGF-β may be involved in enhancing acute inflammatory events. It should be emphasized that these net biologic effects are secondary to multiple cytokines acting in both synergistic and opposing fashions, and the state of differentiation of potential target cells for growth factors influences the resultant biologic response.

Immunoregulatory Cytokines

Interleukins 2, 4, 5, 7, 9, 10, and 11 and IFN-γ are produced by T-cell subsets during an immune response, and they exert effects primarily on that response. In addition, IL-4, IL-10, and IFN-γ exhibit important effects on monocytes and macrophages. Other recently described immunoregulatory cytokines include interleukins 12–18.

T helper (Th) cells are divided into two subsets: Th1 cells produce IFN-γ, IL-2, TNF-α, and IL-1; Th2 cells secrete IL-4, IL-5, and IL-10. In part, these cytokines function to regulate differentiation of T-cell subsets. Th1-cell differentiation is enhanced by IFN-γ and IL-12, the latter of which is produced primarily by macrophages and NK cells. However, Th1-cell differentiation is suppressed by IL-4 and IL-10. In contrast, Th2-cell differentiation is enhanced by IL-4 and inhibited by IFN-γ and IL-12.

Macrophage presentation of processed antigen in complex with a class II MHC molecule stimulates IL-2 production by $CD4^+$ helper T cells. Interleukin 2 then binds to a specific two-chain receptor on target cells in the immediate microenvironment. Interleukin 2 induces a clonal expansion of T cells, enhances B-cell growth, augments NK-cell function, and activates macrophages.

Interleukin 2 production originally was thought to be deficient in people with such autoimmune diseases as RA and systemic lupus erythematosus (SLE). However, these observations may represent an in vitro artifact, and IL-2 production actually may be excessive in these diseases in vivo. The administration of monoclonal antibodies to the IL-2 receptor ameliorates collagen-induced arthritis (CIA) and lupus in mice. This observation argues for the probable importance of IL-2–driven T-cell responses in the counterpart human diseases of RA and SLE.

Soluble IL-2 receptors are found in the circulation of many people with autoimmune, chronic inflammatory, or neoplastic diseases. These receptors probably are released by activated T cells, and their circulating levels correlate with clinical disease activity in some diseases, including SLE. However, these molecules do not inhibit IL-2 in vivo and their presence in circulation merely reflects T-cell activation.

Like Th2 cells, mast cells produce IL-4 (5). Interleukin 4 exerts a major influence on B cells by enhancing IgG_1 and IgE production, inducing the expression of Fc receptors for IgE, and stimulating the expression of class II MHC molecules. Interleukin 4 exhibits stimulatory and suppressive effects on mononuclear phagocytes, again illustrating the principle that a single cytokine may produce mixed or opposing consequences. Interleukin 4 enhances the ability of these cells to present antigen by inducing the expression of class II MHC molecules. Paradoxically, IL-4 directly inhibits monocyte production of IL-1, IL-6, and TNF-α at the level of transcription; these effects of IL-4 potentially are quite anti-inflammatory.

Interleukin 10 and IL-13 function similarly to IL-4 in suppressing monocyte function, although IL-13 is present only in low amounts in the rheumatoid synovium (6,7). Further anti-inflammatory effects of IL-4, IL-10, and IL-13 include the induction of IL-1Ra production. Interleukin 4 also inhibits the production of tissue-degrading neutral metalloproteinases by rheumatoid synovial cells in vitro. Administration of IL-4, either by recombinant-protein injection or gene therapy, is markedly inhibitory to cartilage and bone destruction in CIA in mice (8). IL-4–induced signal transduction molecules are up-regulated in the rheumatoid synovium, although IL-4 protein is present only in small amounts (9). Thus, IL-4 may be exerting anti-inflammatory effects in this tissue, but the cytokine balance remains in favor of the proinflammatory cytokines such as IL-1 and TNF-α.

Interleukins 5, 7, 9, 11, 14, 15, and 16 function primarily as growth and differentiation factors. Interleukin 5 is produced by T cells and enhances the immune response through effects on T and B cells. IL-5 increases IL-2 receptor expression on these cells and promotes antibody secretion by B cells. In addition, IL-5 is the most active known cytokine on eosinophils, inducing chemotaxis, enhancing growth, and stimulating superoxide production. Interleukin 14 is a potent growth factor for B cells.

Interleukins 7, 9, 11, 15, and 16 are primarily growth factors for T lymphocytes. IL-7 and IL-11 are synthesized by bone marrow stromal cells and exhibit additional effects on B cells and hematopoietic cells. Interleukin 7 is a requisite factor for IL-1–induced thymocyte proliferation. These cytokines

influence the function of other cells in addition to T lymphocytes. Interleukin 9 induces proliferation of mast cells; IL-11 synergizes with IL-3 stimulation of megakaryocytes; IL-11, like IL-1 and IL-6, induces the hepatic synthesis of acute-phase proteins; and IL-11 enhances antigen-specific B-cell responses. IL-15 shares biologic properties with IL-2 and is present in high concentrations in RA joints, where it may be responsible for attracting and activating T cells. IL-16 is a chemoattractant for $CD4^+$ T cells.

Unlike IL-2, most immunoregulatory cytokines have not been directly incriminated in pathophysiologic events in rheumatic diseases. However, these cytokines are involved indirectly through their effects on T lymphocytes and other cells. Endogenous IL-4 and IL-5 may play a role in asthma, and endogenous IL-4, IL-10, and IL-13 may dampen synovitis in RA.

Interleukin 17 is a recently characterized cytokine that exhibits biologic functions relevant to joint diseases (Table 4B-3). IL-17 is produced by activated memory $CD4^+$ T cells, and this protein and mRNA are found in large amounts in rheumatoid synovium (10). IL-17 exhibits many proinflammatory effects, including induction of IL-1 and TNF-α production by human macrophages, stimulation of metalloproteinase (MMP)-1 and MMP-9 production by synovial cells, enhancement of nitric oxide production by cartilage, and stimulation of osteoclast differentiation. A single injection of IL-17 into the knees of normal rabbits led to proteoglycan degradation, similar to the effects of IL-1. Inhibition of the production or effects of IL-17 is a potential therapeutic strategy in RA.

Interleukin 18 is another new cytokine that may be important in human disease, particularly in RA as an inducer of the chronic Th1 response present in the synovium (Table 4B-3) (11). The combination of IL-18 with IL-12 or IL-15 induces IFN-γ production in Th1 and NK cells. However, IL-18 exhibits other proinflammatory effects, such as enhancement of GM-CSF, IL-1, and TNF-α production by synovial-lining macrophages. Elevated levels of IL-18 protein and mRNA are present in the rheumatoid synovium, with production by macrophages, chondrocytes, and osteoblasts. A natural inhibitor of IL-18, the IL-18 binding protein, recently has been described and currently is being examined for effects in animal models of arthritis.

IFN-γ is produced simultaneously with IL-2 by antigen-stimulated T cells. A major function of IFN-γ is to enhance antigen presentation by stimulating the expression of MHC class I and II molecules on macrophages, endothelial cells, fibroblasts, and other more tissue-specific cells. IFN-γ also is a potent activator of macrophages, cytotoxic T cells, and NK cells. In addition, IFN-γ stimulates antibody production by B cells but, paradoxically, opposes the effects of IL-4 on these cells. This paradox is an example of self-regulation of the cytokine network through opposing effects.

IFN-γ may be relevant to many human autoimmune diseases, particularly SLE and RA. In SLE, the poor production of IFN-γ by T cells in vitro and the weak response to IFN-γ may reflect cells exhausted by intense IFN-γ effects in vivo, similar to IL-2. In RA, IFN-γ could antagonize the stimulatory effects of TNF-α on many functions of synovial fibroblasts. IFN-γ production probably is deficient in the

TABLE 4B-3
Roles of Interleukins 17 and 18 in Inflammatory Joint Disease

Interleukin 17
- Produced by activated memory $CD4^+$ T cells
- Protein and mRNA present in high levels in the rheumatoid synovium
- Stimulates IL-1 and TNF-α production in human monocytes and macrophages
- Induces MMP production by synovial fibroblasts
- Enhances nitric oxide production by chondrocytes
- Decreases chondrocyte proliferation and proteoglycan synthesis
- Stimulates osteoclast differentiation into bone-resorbing cells

Interleukin 18
- Produced by macrophages, keratinocytes, chondrocytes, fibroblasts, and osteoblasts
- Production stimulated by IL-1 and TNF-α
- Protein and mRNA present in high levels in the rheumatoid synovium
- Stimulates IFN-γ production by Th1 and NK cells, in combination with IL-12 and IL-15, to promote a Th1 phenotype
- Induces GM-CSF, IL-1, and TNF-α production by synovial macrophages
- Stimulates nitric oxide production by synovial cells
- Relative effects in vivo may be counteracted by IL-18 binding protein, a naturally occurring inhibitor

IL, interleukin; TNF, tumor necrosis factor; MMP, metalloproteinase; IFN, interferon; Th, T helper; NK, natural killer; GM-CSF, granulocyte-macrophage colony-stimulating factor.

rheumatoid synovium, predisposing to unregulated TNF-α effects. However, the therapeutic administration of IFN-γ in RA patients appears to offer only modest benefit.

IL-10 is overproduced in SLE, RA, and Sjögren's syndrome and may be involved in some of the pathophysiologic events in these diseases. IL-10 may be involved in enhancing autoantibody production and in inhibiting T-cell function in people with SLE. First- and second-degree relatives of people with SLE demonstrate markedly increased IL-10 production by peripheral B cells and monocytes, in comparison with controls, further implicating excess IL-10 as one predisposing factor in this disease (12). Anti-IL-10 treatment prevents and ameliorates murine models of SLE and appears to have beneficial effects in people with SLE (13).

Thus, the immunoregulatory cytokines may be important in rheumatic disease for their effects on immune cells and macrophages. The possibility of manipulating this group of cytokines for therapeutic advantage has yet to be completely explored.

Proinflammatory Cytokines

The last group of cytokines to be discussed includes TNF-α and interleukins 1, 6, and 8. The functions of these molecules in normal physiology remain unclear, but an understanding of their possible role as inadvertent mediators of inflammation and tissue necrosis continues to grow. TNF-α and IL-1 usually are produced together and may act separately or together in different diseases. The roles of IL-1 and TNF-α in RA have assumed increasing importance with the successful development of therapeutic agents that inhibit their effects (14–16).

TNF-α and TNF-β are related molecules that share the same receptors on the plasma membranes of target cells. TNF-α structurally resembles a transmembrane molecule, and 1%–2% of TNF-α produced resides in the plasma membrane. TNF-α is produced by monocytes, macrophages, lymphocytes, and a variety of transformed cell lines. TNF-α production is stimulated by endotoxin, viruses, and other cytokines. TNF-α receptors p55 and p75 are present on a variety of target cells, and the extracellular portions of these receptors can be cleaved, probably by proteases, releasing soluble receptors. Endogenously produced soluble TNF receptors may function as regulators of extracellular TNF-α effects.

TNF-α exhibits many biologic properties that may be relevant to rheumatic diseases. Along with IL-1, TNF-a induces collagenase and prostaglandin E_2 (PGE_2) production in synovial fibroblasts. TNF-α is present in RA synovial tissues and may be an important inducer of IL-1 in this disease. TNF-α also may induce muscle breakdown and has been associated with the cachexia of congestive heart failure and other chronic diseases. In addition, TNF-α may play pathophysiologic roles in sepsis syndrome and in acute respiratory distress syndrome.

The IL-1 family has at least three known members: two proinflammatory agonists, IL-1α and IL-1β, and a specific naturally occurring inhibitor, IL-1Ra. The two agonists bind to the same receptors and generally produce the same biologic responses, whereas IL-1Ra competitively inhibits receptor binding of IL-1 (14). IL-1α and IL-1β primarily are products of monocytes and macrophages but may also may be produced by endothelial cells, epithelial cells, fibroblasts, activated T cells, and numerous other cells. In humans, IL-1β is the major extracellular product, whereas IL-1α remains primarily membrane-bound.

Two different IL-1 receptors exist. Type I receptors are present on T cells, endothelial cells, and fibroblasts, and type II receptors predominate on B cells, monocytes, and neutrophils (14). Only type I IL-1 receptors are functionally active, whereas type II IL-1 receptors are not capable of inducing intracellular signals. Target cells are exquisitely sensitive to small concentrations of IL-1; occupancy of only 1%–2% of available type I IL-1 receptors stimulates a cell to display complete biologic responses. The expression of type I IL-1 receptors can be down-regulated by TGF-β, partially explaining the immunosuppressive properties of this cytokine.

IL-1 exhibits systemic and local biologic effects in acute and chronic inflammatory disease. Some systemic effects of IL-1 include fever, muscle breakdown and, like IL-6 and IL-11, induction of acute-phase proteins in the liver. The local effects of IL-1 are important in the pathophysiology of joint disease in RA (15). Early in this disease process, IL-1 may enhance expression of adhesion molecules on endothelial cells and induce chemotaxis of neutrophils, monocytes, and lymphocytes into the synovium. Furthermore, IL-1 may contribute to tissue destruction in the rheumatoid joint by inducing PGE_2 and collagenase production by synovial fibroblasts and by chondrocytes present in the articular cartilage. IL-1 may exert similar effects on fibroblasts in other immune and inflammatory diseases, producing tissue damage in lungs, kidneys, or other organs.

IL-6 and IL-8 also play important roles in acute inflammatory diseases. IL-6 is produced in many cells, including synovial cells stimulated by IL-1 or TNF-α. The major functions of IL-6 probably are to induce hepatic synthesis of acute-phase proteins and to enhance Ig synthesis by B cells (17). High levels of IL-6 are present in inflammatory synovial fluids, and IL-6 is present in fibroblasts found in the synovium of RA patients. However, in RA, IL-6 does not induce collagenase and PGE_2 production by synovial fibroblasts and actually may enhance synthesis of a collagenase inhibitor. Interleukin 6 may be primarily responsible for the hypergammaglobulinemia that characterizes many chronic inflammatory diseases.

Interleukin 8 is one member of a family of chemotactic peptides (18). TNF-α and IL-1 stimulate IL-8 production by monocytes, macrophages, endothelial cells, fibroblasts, and other cells. IL-8 is an extremely potent chemotactic factor for neutrophils and may be responsible for attracting these cells into the joint in RA, gout, and other forms of inflammatory arthritis. In addition, IL-8 enhances other neutrophil properties, including expression of adhesion molecules, generation of oxygen radicals, and release of lysosomal enzymes. Thus,

IL-8 may contribute to rheumatic diseases by calling neutrophils into sites of acute inflammation and by activating these cells into an enhanced destructive profile. Other chemokines may be involved in the migration of monocytes into inflamed joints, such as macrophage inflammatory protein-1α and monocyte chemoattractant protein 1.

Regulation of Cytokine Effects

As emphasized throughout this chapter, the cytokine network functions in a self-regulatory fashion. Five different mechanisms regulate the actions of cytokines: specific receptor antagonists, soluble cytokine receptors, antibodies to cytokines, opposing actions of different cytokines, and protein binding of cytokines. Cytokine-inhibiting therapeutic agents would appear to have an important effect in RA (Table 4B-4).

A specific receptor antagonist of IL-1 originally was described in the supernatants of monocytes cultured on adherent IgG and in the urine of febrile patients (19). IL-1Ra is related structurally to IL-1 and binds to both types of human IL-1 receptors on a variety of target cells without inducing discernable biologic responses. IL-1Ra represents the first known naturally occurring molecule that functions as a specific receptor antagonist.

A secreted form of IL-1Ra is produced by monocytes, macrophages, and neutrophils. An intracellular variant of IL-1Ra (icIL-1Ra), which lacks the structural characteristics that lead to secretion, is produced by keratinocytes and other epithelial cells. Additional forms of icIL-1Ra have been described in macrophages, neutrophils, and other cells; it remains unknown whether these molecules perform unique functions inside cells. Alveolar and synovial macrophages secrete little IL-1β, but synthesize large amounts of IL-1Ra, particularly under the influence of GM-CSF. Thus, IL-1Ra is a major product of tissue macrophages and may offer significant antagonism to IL-1 in the pericellular microenvironment of inflammatory tissues.

IL-1Ra has been extensively evaluated in vitro and in vivo, and this molecule blocks the inflammatory effects of IL-1 in every system evaluated. Most importantly, IL-1Ra does not affect normal T- or B-cell responses in vitro or in vivo, suggesting that IL-1 is not required for these responses. IL-1Ra has shown beneficial effects in many animal and in vitro models of human disease, including RA, septic shock, graft-versus-host disease, inflammatory bowel disease, chronic myelogenous leukemia, diabetes mellitus, and asthma. However, very high concentrations of IL-1Ra are required to block the effects of IL-1 in vivo, and IL-1Ra was found ineffective for treating people with sepsis syndrome.

Clinical trials in people with RA indicate that the daily subcutaneous administration of recombinant human IL-1Ra is clinically efficacious and safe. In a six-month trial, 43% of patients experienced a 20% American College of Rheumatology (ACR) response to IL-1Ra administration, compared with 27% in the placebo group (20). Furthermore, treatment with IL-1Ra for six or 12 months reduced radiologic evidence of progression of both joint-space narrowing and bone erosion (21). IL-1Ra also has been delivered successfully by gene therapy in early feasibility trials in RA. A clinical trial in RA examining the simultaneous administration of agents to inhibit both IL-1 and TNF-α is in progress.

Soluble cytokine receptors offer another possible mechanism to inhibit the cytokine network in vivo by binding cytokines in solution and blocking their interaction with target cells. A truncated form of the type I IL-1 receptor was genetically engineered, but it was not effective as a therapeutic agent in RA. Both types of TNF receptors occur in soluble forms and may be effective in blocking TNF effects in vitro and in vivo. Other soluble cytokine receptors have been described, but their in vivo relevance remains unclear.

A therapeutic agent containing the extracellular portion of the p75 TNF receptor coupled to the Fc portion of human IgG1, called etanercept, has proved effective and safe in RA. After six months of twice-weekly subcutaneous injections of this soluble TNF receptor, 20% ACR responses were observed in 59% of treated patients, compared with 11% in controls receiving placebo injections (22). Furthermore, in patients poorly responsive to methotrexate alone, ACR 20% responses were observed in 71% of patients receiving a combination of etanercept and methotrexate for six months, compared with 27% of patients treated with methotrexate alone (23).

Antibodies to many cytokines have been described in the serum of normal individuals, including antibodies to IL-1α (but not IL-1β), TNF-α, and IL-6. These antibodies appear to block the biologic effects of some cytokines, but their in vivo relevance has not been established. A chimeric murine/human monoclonal antibody to TNF-α, called infliximab, has been shown to be quite effective in the treatment of RA. This agent was administered by intravenous

TABLE 4B-4
Inhibition of Cytokine Effects in RA

Agents with demonstrated clinical responses
IL-1Ra (anakinra)
Soluble TNF receptors (etanercept)
Monoclonal antibody to TNF-α (infliximab)
Possible future directions
Combination of IL-1Ra and a TNF inhibitor
Administer anti-inflammatory cytokines such as IL-4, IL-10, or IL-13
Block effects of additional inflammatory cytokines
Monoclonal antibody to IL-6, IL-8, or IL-12
Receptor antagonists to IL-8, IL-12, or IL-17
Soluble receptor to IL-15
Specific binding protein to IL-18

IL-1Ra, interleukin-1 receptor antagonist; TNF, tumor necrosis factor.

infusion every one or two months. In people treated with both infliximab and methotrexate for 30 weeks, 50% experienced a 20% ACR response, compared with 20% receiving methotrexate and placebo (24).

Inhibition of TNF in RA also led to a decrease in radiologic progression of the disease after six to 12 months of treatment. Other forms of arthritis responding to anti-TNF therapy include juvenile rheumatoid arthritis (25), psoriatic arthritis (26), and ankylosing spondylitis (27). TNF inhibitors should not be administered to patients with active or latent infections, and the long-term risk of developing malignancies is not known. Up to 15% of patients receiving anti-TNF agents have developed antinuclear antibodies, with a few patients exhibiting a reversible clinical SLE syndrome.

Numerous examples have been given throughout this chapter of the opposing action of different cytokines. TGF-β has some opposing effects to IL-1 and TNF-α, whereas some effects of TGF-β are opposed by IFN-γ and TNF-α. IL-4 and IL-10 block monocyte production of IL-1, TNF-α, IL-6, and other cytokines. Whether these opposing biologic effects of cytokines can be used to treat human diseases has not been determined. Multiple cytokines bind to α2-macroglobulin in circulation; however, many of these cytokines retain partial or full biologic activities. Thus, protein binding of cytokines as a mechanism to inhibit the effects of cytokines in vivo has not been proved.

Conclusion

The field of cytokine biology is in its infancy. New cytokines continue to be discovered, and existing cytokines are found to possess previously unrecognized biologic properties. Cytokines do not exist solely to cause human diseases; rather, they are mediators of normal cellular events. Particular cytokines may cause unwanted consequences in human disease because they are produced in excess or because their effects are unregulated. Interference with the effects of IL-1 or TNF-α has proved efficacious in subsets of people with RA, and anti–IL-10 therapy may be effective in some people with SLE. Additional anticytokine therapies are under development for these and other rheumatic diseases (Table 4B-4).

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MEDIATORS OF INFLAMMATION, TISSUE DESTRUCTION, AND REPAIR
C. The Complement System

The complement system, discovered more than 100 years ago as a bactericidal (lytic) substance, provides an innate defense against microbes and a "complement" to humoral immunity (see references 1–3 for in-depth reviews). It accomplishes this task by depositing on a target and promoting the inflammatory response. The early reaction sequences behave as biologic cascades in which, by limited proteolysis, one component activates the next, producing a rapid and robust amplification of the system. Because of their capacity to injure tissue, nearly one-half of complement proteins function as regulators or inhibitors (4,5).

In the setting of autoantibodies, complement components unwittingly serve as "inappropriately" guided missiles. Many rheumatic diseases are mediated by immune complexes (ICs). The complement system is critical to the normal handling of ICs, but it also can produce inflammation and tissue damage if ICs lodge in inappropriate locations, such as the joints or kidneys. Deficiencies of the activating components predispose the host to infections (6) and, surprisingly, autoimmune diseases, especially systemic lupus erythematosus (SLE) (7). Complement measurements facilitate the diagnosis and management of lupus and related rheumatic diseases. Further, the system's genetic deficiencies provide intriguing clues relative to the etiology of autoimmune syndromes. These issues, plus complement's putative role in clearance of apoptotic cells and tissue injured by ischemia-reperfusion, have led to a renaissance in the study of complement and its role in innate and adaptive immunity.

Complement Discovery and Function

An ancestral version of the complement system emerged more than 600 million years ago as a host defense system, predating adaptive immunity. Complement systems similar to that of mammals have been found in birds, reptiles, amphibians, fish, sharks, and ascidians. Complement was first identified in the 1890s as a heat-labile fraction of serum that assists (complements) a heat-stable fraction (antibody) to produce bacterial lysis. In the 1950s, a second pathway of complement activation was discovered; the alternative pathway, which provides natural (innate) immunity, can identify and destroy foreign elements *without the need for antibody*. The lectin pathway, characterized more recently, is similar to the activation scheme of

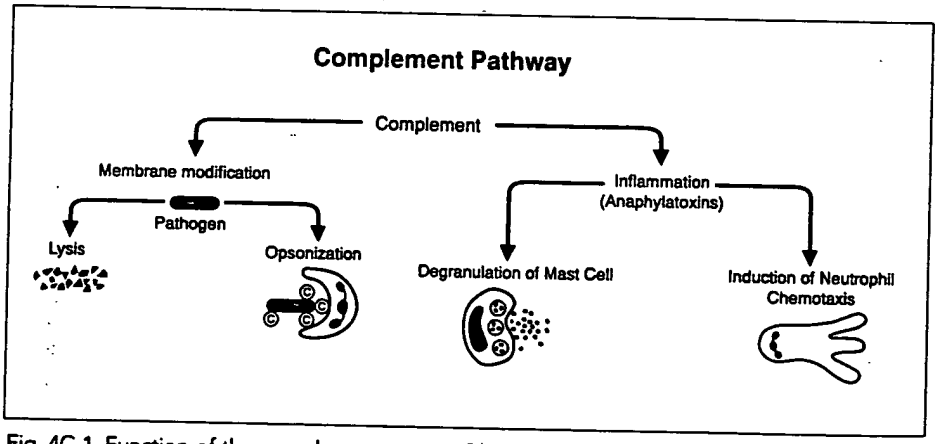

Fig. 4C-1. Function of the complement system. The most important function of complement is to alter the membrane of a pathogen by coating its surface with clusters of complement components (the phenomenon of opsonization). These complements, in turn, facilitate interactions with complement receptors and, in some cases, such as with certain Gram-negative bacteria and viruses, induce lysis. The second function of complement is to promote the inflammatory response. The complement fragments C3a and C5a (called anaphylatoxins) activate many cell types, such as mast cells, to release their contents and phagocytic cells to migrate to an inflammatory site (chemotaxis).